IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Gaetano T. Montelione	:	Confirmation No.: 1781
Application No. 10/534,782	:	Group Art Unit: 1648
Filed: November 16, 2007	:	Examiner: Salimi, Ali Reza
For: PROCESS FOR DESIGNING INHIBITORS OF INFLUENZA VIRUS NON-STRUCTURAL PROTEIN 1	:	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ROBERT M KRUG

1. My name is Robert M. Krug.

2. I received my Ph.D. in 1966 from Rockefeller University. Currently, I am a Professor at Institute for Cellular and Molecular Biology, University of Texas, Austin. The major focus of my laboratory is the molecular biology of human influenza viruses, including viral replication and gene expression, viral-host interactions, and the role of protein modifications in the interferon response. Much of this research centers on the NS1 protein encoded by influenza A viruses (NS1A protein).

3. In course of my career, I authored or co-authored over 110 peer-reviewed articles and two books, mainly devoted to influenza viruses.

4. I read and understood the above-referenced application, including the latest version of the claims submitted to the Examiner with this Declaration.

5. I read and understood the Office Action of October 17, 2008.

6. In the Office Action, the Examiner rejected the claims of the instant application as unpatentable over Wang et al., *Virology*, 233:41 (1996) ("Wang") and Lu et al., *Virology*, 214:222-228 (1995) ("Lu"). The research which is the subject matter of these references was done under my supervision.

7. The Examiner argues that it would be obvious to substitute the 29 bp long dsRNA sequence of Lu and/or the 55 bp long dsRNA sequence of Wang with the 16 bp long dsRNA sequence as claimed in the instant application.

8. Prior to the filing of the instant application (and, by definition, at the time of publication of Wang and/or Lu), the details of molecular interaction between NS1 and dsRNA were unknown, thus making such interactions unpredictable.

9. In my opinion and in view of this unpredictability, a person of ordinary skill in the art would not have reasonably inferred that a short (e.g., about 16 bp long) dsRNA sequence as recited in claim 1 of the instant application would provide sufficiently tight interaction with NS1 or dsRNA binding fragment thereof.

10. More specifically, at the time of filing of the instant application, it was believed that the semi-cooperative binding of multiple NS1 protein molecules to a large dsRNA was required in order to have sufficiently tight binding to detect the protein-dsRNA complexes in gel shift experiments, like those shown in Wang or Lu. The reason for this belief was readily apparent in Wang where it was shown that there was a large decrease in binding affinity when the size of the RNA target was reduced from 140 to 55 bps; and in Lu, where it required a high concentration (4 micromolar) to obtain a gel shift with a 29 bp dsRNA.

11. Further, it was not predictable from Wang or Lu that a small about 16 bp dsRNA could provide sufficiently tight binding to allow a feasible high throughput assay, as discussed in #10 above.

12. The unexpected results of the instant invention provide multiple advantages for the methods and kits utilizing the claimed compositions. Among these advantages are a cheaper manufacturing cost and an opportunity to employ detection methods which are suitable for the high throughput assays.

13. For example, in fluorescence polarization experiments particularly suitable for high throughput assays, the dsRNA is labeled with a fluorescent probe. It was known that binding of the smaller ~ 16 bp dsRNA results in a larger change in rotational correlation time than binding a larger fragment. Accordingly, the amplitude of the signal is much smaller upon complex formation when using a large RNA molecule than when using a smaller ~ 16 bp dsRNA. These issues have been recognized, for example, in Nasir. M.S., Jolley, M.E. Fluorescence polarization: an analytical tool for immunoassay and drug discovery, *Comb. Chem. High Throughput Screen* 2 (1999) 177–190 and in Roehrl, M. H. A., Wang, J. Y., and Wagner, G., A general framework for development and data analysis of competitive high-throughput screens for small-molecule inhibitors of protein-protein interactions by fluorescence polarization. *Biochemistry 43*, 16056-16066 (2004).

I, Robert M. Krug, hereby swear that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willfully false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued hereon.

Date: 1/19/2008

Respectfully Submitted

Robert M. Krug